

Metabolic Labelling (Pulse only or Pulse-chase) (submitted by Martin Kos, Tollervey lab)

Modified from Tollervey *et al.* (1991) *EMBO J.* **10**, 573-583.

- Prepare everything ready and waiting, act quickly.
- Perform pulse chase with only one culture at a time.
- If it is the first time perform a training round of the whole procedure without radioactivity!

Important: Strains used must be URA⁺ or ADE⁺ or MET⁺! If not then transform the strain with an appropriate centromeric plasmid.

Labelling with 3H-uracil or 3H-adenine or 3H-methyle-Methionine

1. Grow cells to OD 0.4 in SD-ura medium
2. Transfer appropriate amount of cells (1 ml per time point) into a pre-warmed Falcon tube or glass flask, grow another ca. 30 min with shaking in an Eppendorf Thermoshaker or water bath.
3. Pulse: add 200 µl/5ml of cell culture of ³H-uracil/³H-adenine or 120 µl ³H methyl-Met
Pulse is 1 min, start the timer at pulse (point 3) to count up
4. Take 1 ml sample and do one of the following
 - a) quick spin (10 sec), discard supernatant and quick freeze the pellet in liquid N₂ or on dry ice/ethanol (this sample is t=0 before the chase, it takes approx. 1 min to process the sample)OR
 - b) freeze directly in 100% ethanol on dry ice (see fast sampling protocol).
5. Chase: after 1 min: add 1/10 volume of 2.4 mg/ml stock cold uracil/adenine or 1/20 volume of 100 mM methionine (preferably prepared in the same growth media as the one used for growing the culture)
6. Take samples at 1 min (just after chase), 2.5 min, 5 min, 10 min or according to your need. Process each sample as before.

RNA extraction according to normal or fast sampling protocol

Separate RNA in an 8% polyacrylamide and 1.2% agarose, transfer to a nylon membrane by **wet electrotransfer** (0.5x TBE, 10V O/N or 20V 5 hours). Expose using a BioMax MS film and BioMax transcreen LE or a phosphorimaging plate (the membrane must be **absolutely dry** otherwise it will damage the imaging plate!). For quantification of large ribosomal RNAs (18S and 25S) it is good to load a very small amount (~0.1 µg total RNA).

Materials:

³H-uracil - Amersham TRK408 (specific activity 1.81TBq/mmol, 49 Ci/mmol)

³H-adenine - Amersham TRK343, as above

³H-methyl-methionine - TRK705 (specific activity 2.66TBq/mmol, 72Ci/mmol)

Note: less radioactive label can be used (e.g. 100 µl/5ml). The sensitivity is decreased, but largely sufficient for traditional pulse-chase.